# Phase II study of radiotherapy and temsirolimus versus radiochemotherapy with temozolomide in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation (EORTC 26082)

Wick, Wolfgang ; Gorlia, Thierry ; Bady, Pierre ; Platten, Michael ; van den Bent, Martin J ; Taphoorn, Martin J B ; Steuve, Jonathan ; Brandes, Alba A ; Hamou, Marie-France ; Wick, Antje ; Kosch, Markus ; Weller, Michael ; Stupp, Roger ; Roth, Patrick ; Golfinopoulos, Vassilis ; Frenel, Jean-Sebastien ; Campone, Mario ; Ricard, Damien ; Marosi, Christine ; Villà, Salvador ; Weyerbrock, Astrid ; Hopkins, Kirsten ; Homicsko, Krisztian ; Lhermitte, Benoit ; Pesce, Gianfranco ; Hegi, Monika E

Abstract: PURPOSE: EORTC 26082 assessed the activity of temsirolimus in patients with newly diagnosed glioblastoma harboring an unmethylated O6 methlyguanine-DNA-methlytransferase (MGMT) promoter. PATIENTS AND METHODS: Patients (n=257) fulfilling eligibility criteria underwent central MGMT testing. Patients with MGMT unmethylated glioblastoma (n=111) were randomized 1:1 between standard chemo-radiotherapy with temozolomide or radiotherapy plus weekly temsirolimus (25 mg). Primary endpoint was overall survival at 12 months (OS12). A positive signal was considered >38 patients alive at 12 months in the per protocol population. A non-comparative reference arm of 54 patients evaluated the assumptions on OS12 in a standard-treated cohort of patients. Pre-specified post hoc analyses of markers reflecting target activation were performed. RESULTS: Both therapies were administered per protocol with a median of 13 cycles of maintenance temsirolimus. Median age was 55 and 58 years in the temsirolimus and standard arms, the WHO performance status 0 or 1 for most patients (95.5%). In the per protocol population, 38 of 54 patients treated with temsirolimus reached OS12. The actuarial 1-year survival was 72.2% [95% CI (58.2-82.2)] in the temozolomide arm and 69.6% [95% CI (55.8-79.9)] in the temsirolimus arm [HR=1.16, 95% CI (0.77-1.76), p=0.47]. In multivariable prognostic analyses of clinical and molecular factors phosphorylation of mTORSer2448 in tumor tissue (HR=0.13, 95% CI (0.04-0.47), p=0.002), detected in 37.6%, was associated with benefit from temsirolimus. CONCLUSIONS: Temsirolimus was not superior to temozolomide in patients with an unmethylated MGMT promoter. Phosphorylation of mTORSer2448 in the pretreatment tumor tissue may define a subgroup benefitting from mTOR inhibition.

DOI: https://doi.org/10.1158/1078-0432.CCR-15-3153

Weller, Michael; Stupp, Roger; Roth, Patrick; Golfinopoulos, Vassilis; Frenel, Jean-Sebastien; Campone, Mario; Ricard, Damien; Marosi, Christine; Villà, Salvador; Weyerbrock, Astrid; Hopkins, Kirsten; Homicsko, Krisztian; Lhermitte, Benoit; Pesce, Gianfranco; Hegi, Monika E (2016). Phase II study of radiotherapy and temsirolimus versus radiochemotherapy with temozolomide in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation (EORTC 26082). Clinical Cancer Research, 22(19):4797-4806.
DOI: https://doi.org/10.1158/1078-0432.CCR-15-3153

Author Manuscript Published OnlineFirst on May 3, 2016; DOI: 10.1158/1078-0432.CCR-15-3153
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

# Phase II study of radiotherapy and temsirolimus versus radiochemotherapy with temozolomide in patients with newly diagnosed glioblastoma without *MGMT* promoter hypermethylation (EORTC 26082)

[1,2]Wolfgang Wick, [3]Thierry Gorlia, [4,5]Pierre Bady, [1,6]Michael Platten, [7]Martin J van den Bent, [8]Martin JB Taphoorn, [3]Jonathan Steuve, [9]Alba A. Brandes, [5,10]Marie-France Hamou, [1]Antje Wick, [11]Markus Kosch, [13]Michael Weller, [10]Roger Stupp, [13]Patrick Roth, [3]Vassilis Golfinopoulos, [12]Jean-Sebastien Frenel, [12]Mario Campone, [14]Damien Ricard, [15]Christine Marosi, [16]Salvador Villa, [17]Astrid Weyerbrock, [18]Kirsten Hopkins, [19]Krisztian Homicsko, [20]Benoit Lhermitte, [21]Gianfranco Pesce, [5,10]Monika E Hegi

[1]Neurology Clinic, University of Heidelberg and [2]Clinical Cooperation Unit (CCU) Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany; [3]European Organisation for Research and Treatment of Cancer (EORTC); [4]SIB Swiss Institute of Bioinformatics, [5]Neuroscience Research Center, University Hospital Lausanne (CHUV), both Lausanne, Switzerland; [6]CCU Brain Tumor Immunology, DKFZ, Heidelberg, Germany; [7]Department of Neurology/Neuro-Oncology, Erasmus MC - Cancer Institute, Rotterdam; [8]Neuro-oncology Unit, MC Haaglanden, The Hague, both The Netherlands; [9]Department of Medical Oncology, Ospedale Bellaria, Bologna, Italy; [10]Department of Neurosurgery, CHUV, Lausanne, Switzerland; [11]Pfizer, Berlin, Germany; [12]1-Institut de Cancérologie de l'OUEST, Saint Herblain-Nantes Cedex, France; [13]Department of Neurology, University Hospital and University of Zurich; [14]AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Neurologie 2-Mazarin; UMR8257 MD4 Cognac G, CNRS, Service de Santé des Armées, Université Paris Descartes, Paris, France; [15]General Hospital AKH, Medical University Vienna, Austria; [16]Institut Catala d'Oncologia (ICO). Hospital Germans Trias Pujol, Badalona, Barcelona, Spain; [17]Department of Neurosurgery, Medical Center - University of Freiburg, Germany; [18]University Hospitals

Bristol NHS Foundation Trust - Bristol Haematology and Oncology Centre, Bristol, U.K.;

[19]Department of Oncology, [20]Institute of Pathology, both CHUV, Lausanne, Switzerland;

[21]Department of Radio-oncology, Oncology Institute of Southern Switzerland, Bellinzona; all Switzerland.

**Running Head:** Temsirolimus for newly diagnosed glioblastoma

**Keywords:** mTOR, biomarker, randomized trial, EORTC, radiochemotherapy, MGMT

**Funding:** Pfizer provided an unrestricted academic grant. Swiss National Science Foundation (FN31003A-138116 to M.E.H) supported the biomarker analyses.

**Prior presentation:** This report has been presented in part as abstract 2003 at ASCO 2014 by W. Wick.

**Trial registration ID:** NCT01019434

**Address correspondence to:** Wolfgang Wick, MD

Neurology Clinic & National Centre for Tumour Disease

University of Heidelberg

Im Neuenheimer Feld 400, D-69120 Heidelberg, Germany

Tel.: +49 (0)6221/56-7075

Fax: +49 (0)6221/56-7554

E-mail: wolfgang.wick@med.uni-heidelberg.de

**Potential conflict of interest:** M.W. has received honoraria from MSD and Merck Serono. W.W. has participated in a speaker's bureau for MSD. W.W. and M.W. have received research funding from MSD. M.W. has received research funding from Merck Serono and

Novocure. W.W. has received research funding from Apogenix, Boehringer Ingelheim, Genentech Roche and Pfizer. R.S. and M.W. have a consultant relationship with MSD and Novocure. M.W. have a consultant relationship with Merck Serono. A.A.B., M.J.v.d.B., P.R., R.S., M.J.B.T., M.W. and W.W. have a consultant relationship with Genentech/Roche. M.K. is an employee of Pfizer, the manufacturer of Temsirolimus.

M.E.H. has served on advisory boards for MSD, Genentech/Roche, and MDxHealth, and has provided services to Novocure.

T.G., P.B., M.P., J.S., M.-F.H., A.W., V.G., J.-S.F., M.C. and B.L. do not have any potential conflicts of interest.

| | |
|---|---|
| Word count | 2996 |
| Figures | 4 |
| Tables | 1 |
| Supplemental Information | |

**Statement of clinical relevance:** The prospective randomized EORTC 26082 trial assessed the tolerability and efficacy of the mechanistic target of rapamycin (mTOR) inhibitor temsirolimus in patients with newly diagnosed, *O6 methlyguanine-DNA-methlytransferase (MGMT)* promoter unmethylated glioblastoma. Temozolomide could be omitted without detriment in the experimental arm. Efficacy of radiotherapy plus temsirolimus failed to reach the pre-specified number of patients alive at 12 months. Pre-specified assessment of activity in the mTOR pathway allows to suggest that one third of patients with phosphorylated mTOR at Ser2448 derive a robust and clinically relevant survival benefit and will be candidates for clinical development of temsirolimus as a targeted therapy in a molecularly defined subgroup.

**ABSTRACT**

**Purpose**: EORTC 26082 assessed the activity of temsirolimus in patients with newly diagnosed glioblastoma harboring an unmethylated O6 methlyguanine-DNA-methlytransferase (*MGMT*) promoter.

**Patients and Methods**: Patients (n=257) fulfilling eligibility criteria underwent central MGMT testing. Patients with *MGMT* unmethylated glioblastoma (n=111) were randomized 1:1 between standard chemo-radiotherapy with temozolomide or radiotherapy plus weekly temsirolimus (25 mg). Primary endpoint was overall survival at 12 months (OS12). A positive signal was considered >38 patients alive at 12 months in the per protocol population. A non-comparative reference arm of 54 patients evaluated the assumptions on OS12 in a standard-treated cohort of patients. Pre-specified post hoc analyses of markers reflecting target activation were performed.

**Results**: Both therapies were administered per protocol with a median of 13 cycles of maintenance temsirolimus. Median age was 55 and 58 years in the temsirolimus and standard arms, the WHO performance status 0 or 1 for most patients (95.5%). In the per protocol population, 38 of 54 patients treated with temsirolimus reached OS12. The actuarial 1-year survival was 72.2% [95% CI (58.2-82.2)] in the temozolomide arm and 69.6% [95% CI (55.8-79.9)] in the temsirolimus arm [HR=1.16, 95% CI (0.77-1.76), p=0.47]. In multivariable prognostic analyses of clinical and molecular factors phosphorylation of mTORSer2448 in tumor tissue (HR=0.13, 95% CI (0.04-0.47), p=0.002), detected in 37.6%, was associated with benefit from temsirolimus.

**Conclusions**: Temsirolimus was not superior to temozolomide in patients with an unmethylated *MGMT* promoter. Phosphorylation of mTORSer2448 in the pretreatment tumor tissue may define a subgroup benefitting from mTOR inhibition.

## INTRODUCTION

The serine/threonine kinase, mechanistic target of rapamycin (mTOR) serves as a hub integrating multiple intra- and extracellular cues in cancer cells (1). mTOR is involved in the formation of two multi-protein complexes, mTORC1 and mTORC2, that direct cell metabolism, growth, proliferation, survival, and angiogenesis.

Preclinical studies suggested an enhanced activity of mTOR inhibition in PTEN-deficient tumour models (2, 3).

Activation of the PI3K/AKT/mTOR pathway has been associated with reduced survival of glioma patients (4) and this signalling pathway has been subjected to a number of negative single- or multi-targeted therapies including the mTOR inhibitor rapamycin or its derivatives, the 'rapalogs' everolimus (RAD001), deforolimus (AP23573), and temsirolimus (CCI-779) (5-9).

The experience with temozolomide (TMZ) teaches that limited activity at recurrence (10) may still relevantly modify the disease in patients with newly diagnosed glioblastoma when combined with radiotherapy (11). Accordingly, mTOR inhibition has been considered an option for patients with treatment-naïve glioblastomas that likely lack some of the mechanisms of resistance acquired at recurrence.

Temsirolimus (Torisel®) has been approved for advanced renal cell carcinoma (12) and relapsed or refractory mantle cell lymphoma (13). Additive effects of temsirolimus plus radiotherapy (RT) in preclinical models demonstrate that temsirolimus could complement the genotoxic activity of RT in the treatment of newly diagnosed glioblastoma. However, combination of TMZ and temsirolimus plus RT was too toxic (14).

Therefore, the rationale of this study was to test the biological effects of mTOR inhibition when combined with ionizing radiation in patients in whom TMZ could be safely omitted. To this end patients with tumors with an unmethylated *O6 methlyguanine-DNA-methlytransferase (MGMT)* gene promoter were selected for the trial, as they derive little if any benefit from the addition of TMZ (15). Another aim was to identify biological factors, i.e.

biomarkers linked to benefit from mTOR inhibition. Temsirolimus may counteract therapy-induced angiogenesis and invasion (16, 17).

## PATIENTS AND METHODS

### *Clinical Trial*

*Study design and treatment*

Patients for EORTC 26082 (NCT01019434) were recruited at 14 study sites in 10 countries in Europe. First, patients were registered after consenting for independent pathology review and central testing of the *MGMT* promoter methylation status by licensed laboratories of MDxHealth (Herstal, Belgium) using quantitative methylation-specific polymerase chain reaction of DNA isolated from macro-dissected formalin fixed paraffin embedded tumor sections (18). Patients were considered *MGMT* unmethylated, applying a safety margin, when the ratio of *MGMT* to the control gene *ACTB* was < 0·6, calculated as (methylated *MGMT*/*ACTB*)×1000. This corresponds to the lower bound of the 95% confidence interval established in a cohort of 602 glioblastoma samples screened in the CENTRIC trial where the cut-off corresponding to the established nadir was at a ratio of 2 that separates methylated from unmethylated. (19) as visualized in **Supplementary Figure S1**. A minimum of 1,250 copies of *ACTB* were required for a valid result, unless the copy number for methylated *MGMT* was ten or more, which was scored as *MGMT* methylated.

Eligible patients (see **Supplementary Information**) were randomly assigned to receive either standard chemoradiotherapy (TMZ/RT→TMZ) (11), or standard fractionated RT with concomitant temsirolimus (standard dose of 25 mg i.v. weekly beginning at day -7 from the start of RT, to be continued until disease progression) (**Figure 1 and Supplement**). The study was conducted according to the Declaration of Helsinki, the International Conference on Harmonisation note for good clinical practice (Topic E6, 1996), and regulatory requirements.

This study was funded by a grant from Pfizer, Berlin, Germany (details on the Role of the Funding Source in the **Supplement**).

*Randomisation and masking*

Randomisation was performed centrally using an interactive voice response system. Patients were stratified according to age, WHO performance status and baseline steroids. As this was an open-label study, no blinding procedures were applied.

*Study endpoints*

The primary endpoint was overall survival at 12 months (OS12) to avoid issues around pseudoprogression and generate a timely signal. Secondary endpoints included progression-free survival (PFS), OS, safety and assessment of prognostic and predictive biomarkers.

*Outcome measures and statistical analyses*

OS12 was defined as the fraction of patients alive at 12 months from randomisation; PFS was defined as duration from randomisation until first observation of PD or death from any cause or censored at last disease assessment without progression or start of second anti-cancer therapy; OS was defined as time from randomisation until death or last visit.

PFS was assessed locally by investigators according to the Macdonald criteria (20), in case of suspected pseudoprogression investigators were advised to continue treatment *per protocol* and repeat imaging after 1-2 months. If progression was confirmed, the date of first observation of tumor progress was used for the analyses.

Adverse events (AEs) were coded according to the Medical Dictionary for Regulatory Activities version 15.0, and their severity was graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

A Fleming one-sample one-stage testing procedure was used in each arm. It was assumed that with OS12 lower or equal to 60% (P0) the therapeutic activity of temsirolimus (CCI-779) was too low(11). While a OS12 greater or equal to 80% (P1) implied that the therapeutic activity of temsirolimus (CCI-779) was adequate Type I (α) and II (β) errors were both equal to 5%. Under these hypotheses, a sample size of 54 eligible patients in each arm was

required. The decision rule was that if >38 eligible patients were alive at 1 year, it was concluded that the therapeutic activity of temsirolimus was adequate.

All statistical analyses were performed on mature data (median follow-up 32 months) by Thierry Gorlia. The concept of a non-comparative control arm allows for adjustment of the initial assumptions based on contemporary control treatment. The trial would be insufficient to confirmatory declare efficacy. However, statistical comparisons are still valid and useful for hypothesis-generation and exploratory analyses.

The OS12 was also computed in the TMZ/RT→TMZ arm in order to assess the consistency with P0.

***Biomarker substudy***

*Tissue Micro Array, Immunohistochemistry and FISH EGFR*

Tissue micro arrays (TMA) were constructed using recipient paraffin blocks with an agarose matrix (21). Immunohistochemical analyses and Fluorescent *In Situ* Hybridization (FISH) were performed in duplicate on sections from 2 replicate TMAs basically as recommended by the manufacturers (see supplemental methods for antibody description, conditions and dilutions; FISH probes). Markers for *post hoc* analyzes of the mTOR pathway were pre-specified in the protocol (phosphorylated S6 ribosomal protein, p-S6RP$^{Ser235/236}$; phosphorylated AKT, p-AKT$^{Ser473}$; PTEN; phosphorylated AKT1 Substrate 1 (proline-rich), p-PRAS40$^{Thr246}$; phosphorylated extracellular signal-regulated linase, ERK1/2$^{Thr202/Tyr204}$) or based on a more recent study (phosphorylated p-mTOR$^{Ser2448}$) (22, 23). Scoring and definition of dichotomization is detailed in the Supplemental Methods.

*Multidimensional marker analysis*

The centered score table of the markers containing missing values was analysed by principal component analysis. Non-linear Iterative Partial Least Squares (NIPALS) algorithm (24) was used to perform singular-value decomposition with missing value and to complete

the data. A consensus hierarchical clustering analysis (25) based on Euclidean distance and Ward's algorithm was used to investigate the optimal number of clusters. The association among marker scores was illustrated by network representation based on Spearman correlation. Analyses and graphical representations were performed using R-3.2.0 and the R packages mixOmics, qgraphs (26) and ConsensusClusterPlus.

*Statistical analysis*

The scores of the P-markers were dichotomized into negative (scores 0, 1, corresponding to 0 to10%) vs positive (scores 2 to 5, >10%). Study stratification factors (age, WHO performance status, baseline steroids) and molecular markers were correlated to OS.

Treatment arms were compared with a log-rank test at 5 % significance. For each of them, PFS and OS were estimated using the Kaplan-Meier (KM) method. Associations of marker profiles with treatment efficacy were presented by Forest Plot and significance was assessed with the test for interaction computed from a Cox model including the treatment, the marker and their interaction term. A 5% significance was used for screening predictive markers. For each factor, univariable survival estimates were calculated using the KM technique in the TMZ and temsirolimus arms. Hazard Ratios obtained from univariable Cox models were presented with 95 % Confidence Intervals (CI) (details in the **Supplement**).

## RESULTS

### *Patients*

Overall, 257 patients were registered, screened for eligibility and assessed for *MGMT* promoter methylation status, whereof 28 patients were registered after screening through the CENTRIC trial that selected *MGMT* methylated patients only (19); 190 patients were found to have glioblastoma with an unmethylated *MGMT* promoter applying the cut-off with a safety margin (Figure S1). The primary reasons for initially registered patients not to continue to randomisation were hypermethylated *MGMT* status (n=67), withdrawal of consent (n=24), and other reasons (n=55), including insufficient tumor material (n=30), and AEs after surgery (n=8) **(Figure 1)**. A total of 111 patients were randomised from December 2009 through September 2012 and constituted the ITT population: 56 patients were scheduled to receive weekly temsirolimus in addition to standard RT (temsirolimus arm) and 55 were to receive TMZ/RT→TMZ alone (control arm). In the safety population, i.e. patients with at least one dose of drug, there were 53 patients in the temsirolimus and 51 patients in the TMZ arm.

Median follow-up was 33 (95% CI: 23-37) months in the temsirolimus and 32 (95% CI: 22-40) months in the TMZ arm. The median duration from operation to randomisation was 2.6 weeks (range 0.4–6.1 weeks). Patient baseline and demographic characteristics were well balanced between treatment arms except for the WHO Performance status between PS0 and PS1, which favored the control arm. This is explained since the stratification was PS 0-1 *vs* PS2 (**Table 1**).

In the biomarker cohort (n=88), only one patient sample displayed positive staining for the IDH1-R132H mutant (1/78; 1.3%), an expected low frequency, since 75% of the few *IDH1* mutant glioblastoma are *MGMT* hypermethylated (27). The frequency of *EGFR* amplification was in the expected range (54%, 44/82). There was no difference in baseline characteristics and outcome in patients with *vs* without markers assessment (**Supplementary Figure S2**,

**Supplementary Table S1**).

***Efficacy outcomes***

The median duration of radiotherapy was 6.1 weeks in both arms. Main reason for interrupting RT was technical or administrative (28%). In median, RT was interrupted 2 days. RT was completed by >90% of patients. Concomitant treatment was delivered as planned *per protocol* by >90% of patients in both arms. Patients in the temsirolimus arm received the drug for a median (95% CI) of 16 weeks post RT (4.0 – 84.3), with a mean dose intensity of 21.4 (6.3 - 25) mg/week.

Maintenance temsirolimus was administered *per protocol* at a median of 13 weekly cycles. Median relative dose-intensity was 85.6%. Twelve patients had a reduction in dose intensity below 70%, because of dose reduction (19.1%: 6.4% for hematological toxicity, 10.6% for AE, 2.1% for other reasons), dose not given during at least one cycle (68%: 6.3% for hematological toxicity, 34% for non-hematological toxicity, 58% for other reasons) or treatment delay (58%: 2.1% for hematological toxicity, 17% for non-hematological toxicity, 43% for other reasons).

Median OS was 14.8 (13.3-16.4) months in the temsirolimus arm and 16.0 (13.8-18.2) in the control arm (90 deaths; HR, 1.2; 95% CI, 0.8-1.8; p=0.47; **Figure 2A**). The OS12 and OS24 rates did not differ between arms (70%, 72% and 15%, 16%, respectively). Median PFS as assessed by the investigator was 5.4 (95% CI, 3.7-6.1) months in the temsirolimus arm and 6.0 (95% CI, 2.8-8.0) months in the control arm (54 PFS events; HR, 1.26; 95% CI, 0.86–1.86; p=0.24; **Figure 2B**). In the *per protocol* population (see **Supplementary Information**), 38 patients treated with temsirolimus had survived ≥ to 1 year. At least 39 patients were needed to reach the targeted drug activity.

***Safety***

In the temsirolimus arm severe hematological toxicity was: neutropenia (G3: n=1, 1.9%) and lymphocytopenia (G3: n=9, 16.4%, G4: n=1, 1.8%). In the TMZ arm severe hematological

toxicity was: leukopenia G3 (n=2, 3.8%), neutropenia G4 (n=2, 3.8%), lymphocytopenia (G3: n=14, 26.4%, G4: n=2, 3.8%) and thrombocytopenia (G3: n=1, 1.9%, G4: n=1, 1.9%). There was no other severe (G3/4) treatment-related AE with an incidence >5% in either arm.

***Molecular correlations with outcome***

Markers interrogated for their relevance of targeting the mTOR signaling pathway (22, 23) are visualized in the mTOR KEGG pathway (28) (**Supplementary Figure S3**). Phosphorylated mTOR$^{Ser2448}$ was associated with prolonged OS as evidenced by the significant interaction term between treatment and p-mTOR$^{Ser2448}$ (p=0.047, **Figure 3**). Tumors of 37.6% of the patients scored positive for p-mTOR$^{Ser2448}$. There was a non-significant trend for longer OS when p-mTOR$^{Ser2448}$ positive patients received temsirolimus as compared with controls (HR=0.62, 95% CI 0.26-1·47, p=0.27). When non-phosphorylated mTOR$^{Ser2448}$ patients received temsirolimus a non-significant decrease in survival was observed compared with controls (HR=1.77, 95% CI 0.95-3.29, p=0.07) (**Figure 3**). The median OS in the temsirolimus group was 17.8 months (CI, 14.1-28.0) for patients with p-mTOR$^{Ser2448}$ positive tumors and 13.1 months (CI, 9.7-15.1) in the negative subgroup (p=0.007, Figure 3A). In the RT/TMZ→TMZ control arm the median OS in the p-mTOR$^{Ser2448}$ positive group was 14.0 months (CI, 9.6-19.6) and 16.5 months (CI, 9.5-18.8) in the p-mTOR$^{Ser2448}$ negative subgroup (p=0.999). For p-PRAS40$^{Thr246}$, the interaction test with treatment was borderline non-significant (p=0.07). The impact of all other markers on survival is illustrated in a forest plot for all other markers in **Supplementary Figure S4**.

A multi dimensional analysis used the full range of the scores of the mTOR-associated markers integrated information for the identification of clinically relevant molecular subgroups and to gain further insights on pathway interactions (**Figure 4**). The two first axes obtained by PCA explained 57·8% of the total inertia. The first axis was mainly explained by p-mTOR$^{Ser2448}$ and p-PRAS40$^{Thr246}$. The p-S6RP$^{Ser235/236}$ mainly contributed to the construction of the second axis (**Figures 4E and F**). PTEN expression played a minor role in the

structure of the score table (**Figure 4F**). Subgroups were determined by consensus clustering. We kept the cluster based on two groups (k=2) by default, as no strong indication for the optimal number of clusters was obtained and the sample size is limited (**Supplementary Figure S5**). Cluster 2, highly enriched for p-mTOR$^{Ser2448}$-positive cases, revealed a strong association with outcome in the temsirolimus treatment group and no difference in the TMZ/RT→TMZ group (**Figure 4**). Significant interaction was observed with treatment (p=0.009): in Cluster 2 the HR was 0.42 (95% CI 0.15-1.13, p=0.08) and in Cluster 1 HR=1.77 (95% CI 0.96-3.25, p=0.06).

In multivariable prognostic analyses of clinical and molecular factors (**Supplementary Table S1**), p-mTOR$^{Ser2448}$ (HR=0.13, 95% CI 0.04-0.47, p=0.002), p-PRAS40$^{Thr246}$ (HR=0.50, 95% CI 0.21-1.18, p=0.12), p-ERK$^{Thr202/Tyr204}$ (HR=2.81, 95% CI 0.97-8.09, p=0.06), but no clinical factor was associated with OS in the temsirolimus arm. The PEV was equal to 14.9% In the TMZ arm, there was a trend for decreased survival in p-AKT$^{Ser473}$ positive patients (HR=3.21, 95% CI 0.89-11.56, p=0.07, PEV=4.5%). None of the models had a PEV larger than 20%.

## DISCUSSION

This randomized, open label phase II trial investigating the mTOR inhibitor temsirolimus in combination with RT for patients with low probability of benefit from the TMZ-based radiochemotherapy failed to demonstrate the targeted outcome. Neither PFS nor OS demonstrated a signal of relevant activity in the total trial population (**Figure 2**). Safety and tolerability of temsirolimus in combination with standard RT were non-concerning and the trial is an example that temozolomide can be safely omitted in patients with *MGMT* unmethylated glioblastoma. The trial proposes $mTOR^{Ser2448}$ phosphorylation as a biomarker for benefit from mTOR inhibition. These results need further confirmation, and a trial to prospectively assess the relevance of this putative biomarker is underway (NCT Neuro Master Match, *EudraCT 2015-002752-27*).

The good outcome data in both arms of the trial prompted a comparison with the EORTC26981-22981/NCIC CE3 trial. The comparison with our pivotal TMZ/RT→TMZ vs RT trial (EORTC26981-22981/NCIC CE3) (29) was favourable in all aspects supporting the principal rational to design trials for patients with *MGMT* unmethylated glioblastoma and withhold TMZ in the experimental arm (**Supplementary Results**). Biases in favor of EORTC 26082 may have been patient selection, and the lower number of patients on steroids (30). Bevacizumab was administered in about 45% of the patients in both arms of EORTC 26082. The OS of the EORTC 26082 arms is comparable to the outcome in the control arms of trials with selection of *MGMT* unmethylated patients, with 13.4 months in the CORE trial (95% CI 12.2-14.3) with a bevacizumab use at recurrence of 22% (31) and 17.3 months (95%CI 14.8-20.4 months) in the GLARIUS trial with cross over to bevacizumab of 60% (32).

The EORTC 26082 trial aimed at not withholding TMZ from any patient with an equivocally methylated *MGMT* promoter by applying a *MGMT* cut-off with a safety margin. This prompted an adaption also in the GLARIUS trial (32) with similar design and therefore demarcates an evolution from the S039 trial with enzastaurin (33). Two randomized phase III trials in elderly patients with newly diagnosed glioblastoma further support a strictly

predictive effect of the *MGMT* status for benefit from TMZ (34, 35). However, we cannot completely exclude a small baseline effect of TMZ despite the *MGMT* unmethylated state (11). Hence, withholding TMZ outside trials and elderly patients with unmethlylated *MGMT* promoter is not advocated by the present data. In the temsirolimus arm 59% (n=33) of the patients received TMZ after treatment discontinuation, and 26% of TMZ patients (n=14) were re-challenged with TMZ, not being aware of the recent data from the DIRECTOR trial that re-challenge with TMZ might be relevant only for patients with a methylated *MGMT* promoter (36).

The choice of temsirolimus for patients with unmethylated glioblastoma was based on preclinical data already highlighting that not every tumor responds to the treatment (37) as well as a response may be only transient because of the overt feedback resistance mechanisms (22, 38).

Molecular analyses of prespecified principal components of the EGFR-PI3-K/mTOR/AKT pathway were performed. EORTC 26082 provides first evidence that p-mTOR$^{Ser2448}$ and – to a lesser extent - p-PRAS40$^{Thr246}$ may serve as decisive biomarkers for the treatment of patients with newly diagnosed glioblastoma with an unmethylated *MGMT* promoter. Phosphorylation of mTOR$^{Ser2448}$ has been shown to be targeted and blocked by rapamycin, a major metabolite of temsirolimus (39), while phosphorylated PRAS40$^{Thr246}$ (substrate of AKT1) relieves inhibitory function on mTORC1 (40). The survival curves may even suggest that there is a detrimental effect of temsirolimus in p-mTOR$^{Ser2448}$ negative tumors (**Figures 3 and 4**). Previous trials testing temsirolimus at recurrence had focused on the PTEN status with a PTEN deficiency as a prerequisite for response (22) or on other downstream mTOR targets, e.g. p-S6RP$^{Ser235/236}$, which was neither associated with outcome in biomarker analyses of patients with recurrent glioblastoma receiving temsirolimus (6, 38) nor in this study. It cannot be excluded that glioblastomas treated at recurrence may have changed mTOR pathway activity as compared to tumor specimen used for marker analyses obtained at the first resection (41). Also, “paradoxical” activation of AKT by elimination of negative feedback downregulating survival signaling has been postulated as potential resistance

mechanism to mTOR inhibition in previous trials, based on the analyzes of paired tumor specimen taken before and after treatment (22, 38). Interestingly, trials in other diseases did not provide predictive biomarkers (12, 13).

The limitations of EORTC 26082 are the relatively small sample size of this non-comparative phase II trial. For the biomarker analyses using IHC only a limited number of tumor tissue samples from the ITT cohort were available. The findings should be validated by evaluation of previous trials in particular in those treating newly diagnosed glioblastoma patients (42) and the randomized phase II study RTOG-0913. Ongoing trials using mTOR inhibitors may need to take into account a potentially detrimental effect in patients with an unphosphorylated $mTOR^{Ser2448}$. Given the ongoing efforts of biomarker-driven basket trials for patients with newly diagnosed glioblastoma, the concept of mTOR inhibition using the marker predictive in this study, $p\text{-}mTOR^{Ser2448}$ is incorporated into the design of a future study.

411 **REFERENCES**

412

413 1. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR*, et*
414 *al.* The somatic genomic landscape of glioblastoma. Cell 2013;155: 462-77.
415 2. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R*, et al.* Enhanced
416 sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci U S A
417 2001;98: 10314-9.
418 3. Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J*, et al.* An inhibitor of
419 mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/- mice. Proc Natl
420 Acad Sci U S A 2001;98: 10320-5.
421 4. Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A*, et al.* The
422 prognostic significance of phosphatidylinositol 3-kinase pathway activation in human
423 gliomas. J Clin Oncol 2004;22: 1926-33.
424 5. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as
425 anticancer agents. Nat Rev Drug Discov 2006;5: 671-88.
426 6. Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J*, et al.* Phase II
427 trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer
428 Treatment Group Study. J Clin Oncol 2005;23: 5294-304.
429 7. Doherty L, Gigas DC, Kesari S, Drappatz J, Kim R, Zimmerman J*, et al.* Pilot study of
430 the combination of EGFR and mTOR inhibitors in recurrent malignant gliomas. Neurology
431 2006;67: 156-8.
432 8. Kreisl TN, Lassman AB, Mischel PS, Rosen N, Scher HI, Teruya-Feldstein J*, et al.* A
433 pilot study of everolimus and gefitinib in the treatment of recurrent glioblastoma (GBM). J
434 Neurooncol 2009;92: 99-105.
435 9. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Friedman AH, Herndon
436 JE, 2nd*, et al.* Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. J
437 Neurooncol 2010;96: 219-30.

10. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD*, et al.* A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. Br J Cancer 2000;83: 588-93.

11. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB*, et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352: 987-96.

12. Motzer RJ, Hudes GR, Curti BD, McDermott DF, Escudier BJ, Negrier S*, et al.* Phase I/II trial of temsirolimus combined with interferon alfa for advanced renal cell carcinoma. J Clin Oncol 2007;25: 3958-64.

13. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C*, et al.* Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol 2009;27: 3822-9.

14. Sarkaria JN, Galanis E, Wu W, Dietz AB, Kaufmann TJ, Gustafson MP*, et al.* Combination of temsirolimus (CCI-779) with chemoradiation in newly diagnosed glioblastoma multiforme (GBM) (NCCTG trial N027D) is associated with increased infectious risks. Clin Cancer Res 2010;16: 5573-80.

15. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M*, et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352: 997-1003.

16. Abdollahi A, Lipson KE, Han X, Krempien R, Trinh T, Weber KJ*, et al.* SU5416 and SU6668 attenuate the angiogenic effects of radiation-induced tumor cell growth factor production and amplify the direct anti-endothelial action of radiation in vitro. Cancer Res 2003;63: 3755-63.

17. Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001;61: 2744-50.

18. Vlassenbroeck I, Califice S, Diserens AC, Migliavacca E, Straub J, Di Stefano I*, et al.* Validation of real-time methylation-specific PCR to determine O6-methylguanine-DNA methyltransferase gene promoter methylation in glioma. J Mol Diagn 2008;10: 332-7.

19. Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong YK*, et al.* Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 2014;15: 1100-8.

20. Macdonald DR, Cascino TL, Schold SC, Jr., Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 1990;8: 1277-80.

21. Yan P, Seelentag W, Bachmann A, Bosman FT. An agarose matrix facilitates sectioning of tissue microarray blocks. J Histochem Cytochem 2007;55: 21-4.

22. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S*, et al.* Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. PLoS Med 2008;5: e8.

23. Hegi ME, Diserens AC, Bady P, Kamoshima Y, Kouwenhoven MC, Delorenzi M*, et al.* Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib - A phase II trial. Mol Cancer Ther 2011;10: 1102-12.

24. Wold H. Estimation of principal components and related models by iterative least squares. Multivariate Analysis: Academic Press; 1966. p. 391-420.

25. Monti S, Tamayo P, Mesirov J, Golub T. Consensus clustering: A resampling-based method for class discovery and visualization of gene expression microarray data. Machine Learning 2003;52: 91-118.

26. Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D. qgraph: Network visualizations of relationships in psychometric data. J Stat Soft 2012;48: 1-18.

27. Bady P, Sciuscio D, Diserens AC, Bloch J, van den Bent MJ, Marosi C*, et al.* MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction

model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol 2012;124: 547-60.

28. Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics 2013;29: 1830-1.

29. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC*, et al.* Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10: 459-66.

30. Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC*, et al.* Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. Lancet Oncol 2014;15: 943-53.

31. Nabors LB, Fink KL, Mikkelsen T, Grujicic D, Tarnawski R, Nam DH*, et al.* Two cilengitide regimens in combination with standard treatment for patients with newly diagnosed glioblastoma and unmethylated MGMT gene promoter: results of the open-label, controlled, randomized phase II CORE study. Neuro Oncol 2015;17: 708-17.

32. Herrlinger U, Schäfer N, Steinbach JP, Weyerbrock A, Hau P, Goldbrunner R*, et al.* The randomized, multicenter glarius trial investigating bevacizumab/irinotecan vs standard temozolomide in newly diagnosed, mgmt-non-methylated glioblastoma patients: final survival results and quality of life. Neuro-Oncology 2014;16: ii23-ii4.

33. Wick W, Steinbach JP, Platten M, Hartmann C, Wenz F, von Deimling A*, et al.* Enzastaurin before and concomitant with radiation therapy, followed by enzastaurin maintenance therapy, in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation. Neuro Oncol 2013;15: 1405-12.

34. Wick W, Platten M, Meisner C, Felsberg J, Tabatabai G, Simon M*, et al.* Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. Lancet Oncol 2012;13: 707-15.

518 35. Malmstrom A, Gronberg BH, Marosi C, Stupp R, Frappaz D, Schultz H*, et al.*
519 Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy
520 in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial.
521 Lancet Oncol 2012;13: 916-26.
522 36. Weller M, Tabatabai G, Kastner B, Felsberg J, Steinbach JP, Wick A*, et al.* MGMT
523 promoter methylation is a strong prognostic biomarker for benefit from dose-intensified
524 temozolomide rechallenge in progressive glioblastoma: The DIRECTOR trial. Clin Cancer
525 Res 2015;21: 2057-64.
526 37. Weiler M, Pfenning PN, Thiepold AL, Blaes J, Jestaedt L, Gronych J*, et al.*
527 Suppression of proinvasive RGS4 by mTOR inhibition optimizes glioma treatment.
528 Oncogene 2013;32: 1099-109.
529 38. Wen PY, Chang SM, Lamborn KR, Kuhn JG, Norden AD, Cloughesy TF*, et al.* Phase
530 I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas: North
531 American Brain Tumor Consortium trial 04-02. Neuro Oncol 2014;16: 567-78.
532 39. Chiang GG, Abraham RT. Phosphorylation of mammalian target of rapamycin
533 (mTOR) at Ser-2448 is mediated by p70S6 kinase. J Biol Chem 2005;280: 25485-90.
534 40. Wiza C, Nascimento EB, Ouwens DM. Role of PRAS40 in Akt and mTOR signaling
535 in health and disease. Am J Physiol Endocrinol Metab 2012;302: E1453-60.
536 41. Kim H, Zheng S, Amini SS, Virk SM, Mikkelsen T, Brat DJ*, et al.* Whole-genome and
537 multisector exome sequencing of primary and post-treatment glioblastoma reveals patterns
538 of tumor evolution. Genome Res 2015;3: 114.
539 42. Ma DJ, Galanis E, Anderson SK, Schiff D, Kaufmann TJ, Peller PJ*, et al.* A phase II
540 trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed
541 glioblastoma: NCCTG N057K. Neuro Oncol 2015;17: 1261-9.
542
543
544

## ACKNOWLEDGMENTS

We are indebted to the patients and their families for agreeing to participate in this trial, as well as to the nurses and data managers for their collaboration. A list of the participating investigators is provided in the **Supplement**.

Pfizer provided an unrestricted academic grant. Molecular subgroup analysis was funded by the Swiss National Science Foundation (FN31003A-138116 to M.E.H).

This report has been presented in part as abstract 2003 at ASCO 2014 by W. Wick.

## CONTRIBUTORS

The concept of the trial was developed by W.W. in collaboration with the T.G., G.P., M.E.H., R.S. and the EORTC Brain Tumor Group. The concept of the biomarker analyses was developed by M.E.H. in collaboration with T.G, P.B. and W.W.

Study material: W.W., M.P., M.J.v.d.B., M.J.B.T., A.A., M.W., P.R., M.C., J.-S. F., M.W., R.S., D.R., C.M., S.V., A.W., Ki.H., Kr.H., G.P. recruited patients to the study, were involved in data collection and provided administrative support.

The biomarker data were generated and evaluated by P.B., M.-F.H, B.L. and M.E.H. Reference pathology was performed by B.L.

The statistical analyses were performed by T.G. and P.B.

The article was written by W.W. and M.E.H. with support from all co-authors.

All authors reviewed and approved the manuscript.

## FIGURE LEGENDS

**Figure 1. Supplemented CONSORT diagram of patient disposition.**

**Figure 2. Principal efficacy outcomes per treatment.**

**Figure 3. Overall survival according to phosphorylated mTOR stratified by treatment.**

(**A**) Kaplan-Meier curves shown represent patients separated by the phosphorylation status of $mTOR^{Ser2448}$ (Pos, positive; Neg, negative) stratified for the two treatment arms CCI-779/RT and TMZ/RT→TMZ (TMZ). The interaction test was significant p=0.047). (**B**) Representative glioblastoma samples negative or positive for p-$mTOR^{Ser2448}$ expression.

**Figure 4. Multidimensional analysis of m-TOR associated markers.**

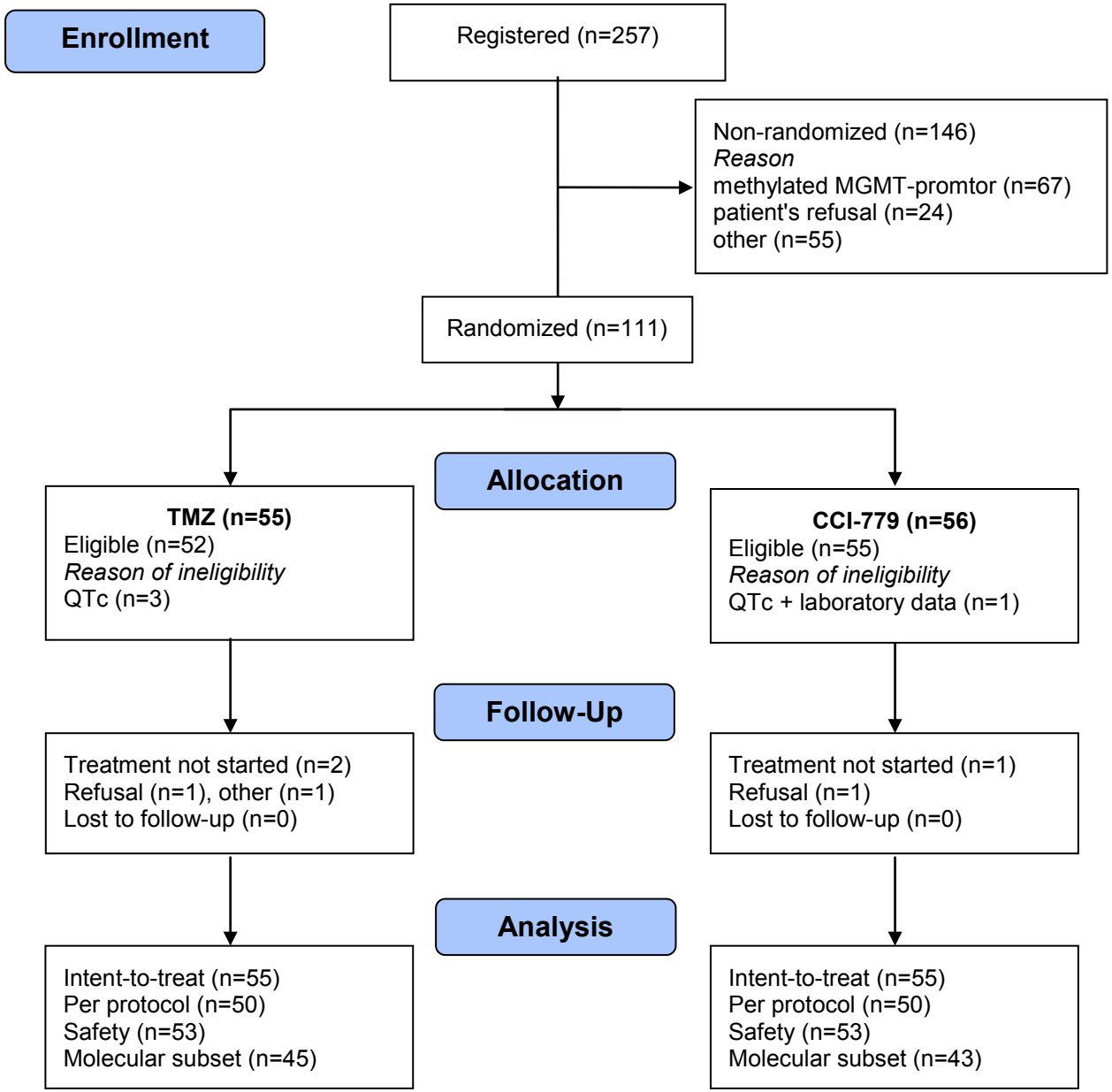
The associations among markers in the mTOR pathway are illustrated by "The network representation" based on Spearman correlations between scores (**A**). (**B)** The glioblastoma subgroups based on mTOR pathway markers are visualized in a heatmap of the score table obtained after reconstruction using Non-linear Iterative Partial Least Squares (NIPALS). The rows were ordered by the first axis of the PCA. The columns are ordered by the consensus classification (k=2; clusters 1, blue; cluster 2, red) and are annotated for absence or presence of mutated $IDH1^{R132H}$ (positive, red; negative, grey; unknown; white), and the *EGFR* status (amplified dark green, non-amplified, green; unknown, white). The association between OS and consensus classification for two groups (k=2) (cluster 1, blue; cluster 2, red) is illustrated by Kaplan-Meier representation for patients randomized to CCI-779 **(C)** and TMZ **(D)**. The p-value is given for each KM. The patients **(E)** and m-TOR-associated markers **(F)** were projected onto the two first components of the principal component analysis (PCA). Inertia ellipses and stars visualize the separation of the patients into the two groups obtained from consensus clustering (cluster 1, blue; cluster 2, red) **(E)**.

**Table Baseline characteristics**

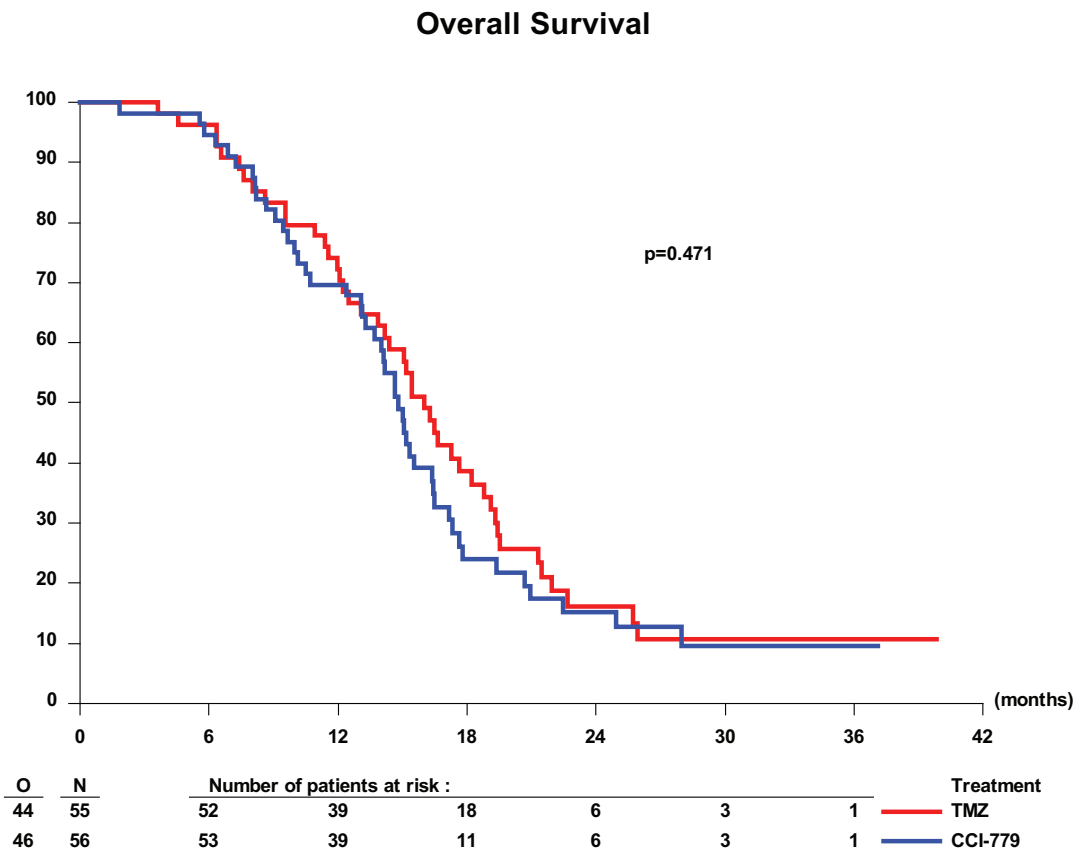
| | TMZ (N=55) | Temsirolimus (N=56) | Total (N=111) |
|---|---|---|---|
| | N (%) | N (%) | N (%) |
| **Age** | | | |
| **median** | 57.7 | 54.9 | 55.7 |
| **range** | 24.4 - 76.0 | 28.2 - 74.7 | 24.4 - 76.0 |
| **Sex** | | | |
| **male** | 36 (65.5) | 35 (62.5) | 71 (64.0) |
| **female** | 19 (34.5) | 21 (37.5) | 40 (36.0) |
| **Extent of resection** | | | |
| **open biopsy** | 1 (1.8) | 3 (5.4) | 4 (3.6) |
| **resection** | 54 (98.2) | 53 (94.6) | 107 (96.4) |
| **Corticosteroids** | | | |
| **no** | 37 (67.3) | 40 (71.4) | 77 (69.4) |
| **yes** | 18 (32.7) | 16 (28.6) | 33 (29.7) |
| **WHO PS (0-4)** | | | |
| **0** | 40 (72.7) | 32 (57.1) | 72 (64.9) |
| **1** | 14 (25.5) | 20 (35.7) | 34 (30.6) |
| **2** | 1 (1.8) | 4 (7.1) | 5 (4.5) |

Abbreviations: TMZ, temozolomide; WHO PS, World Health Organization Performance Status

Figure 1



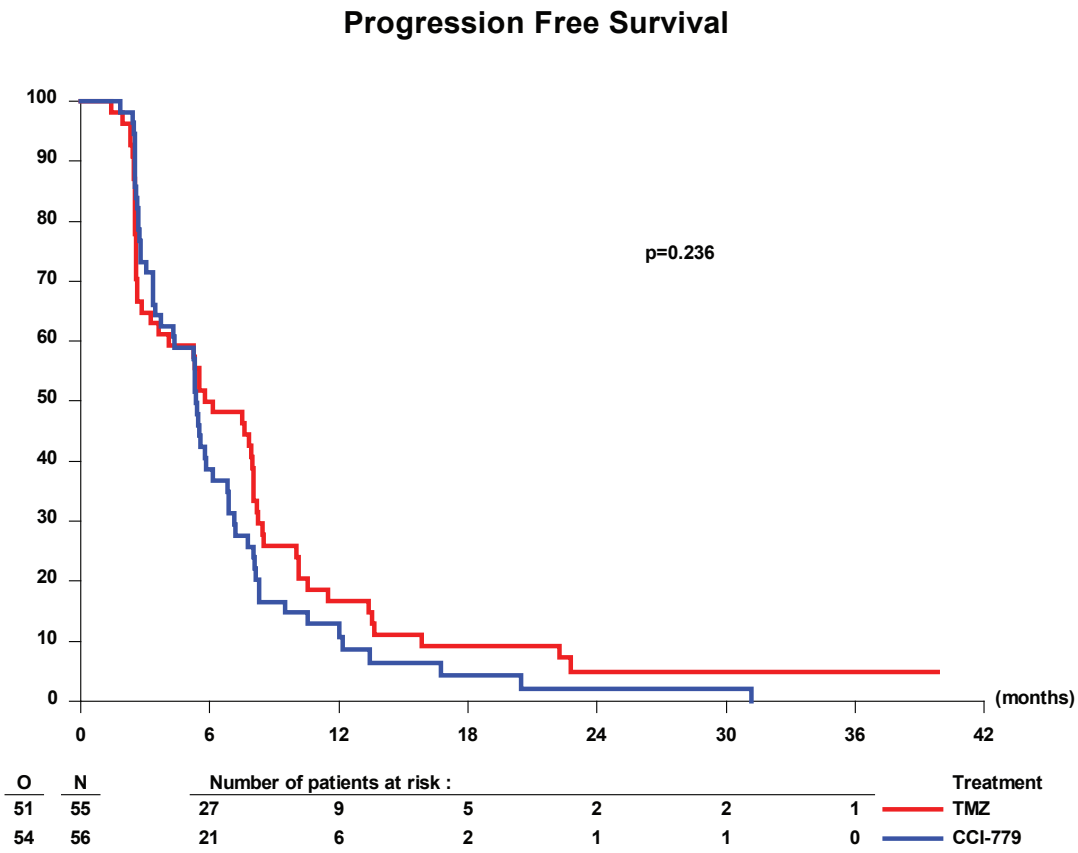
A




## Survival Time

| Treatment | Patients (N) | Observed Events (O) | Hazard Ratio (95% CI) | P-Value (Log-Rank) | Median (95% CI) (Months) | % at 1 Year (95% CI) |
|---|---|---|---|---|---|---|
| **TMZ** | 55 | 44 | 1.00 | 0.4708 | 16.03 (13.83, 18.20) | 72.22 (58.22, 82.22) |
| **CCI-779** | 56 | 46 | 1.16 (0.77, 1.76) | | 14.78 (13.27, 16.39) | 69.64 (55.79, 79.91) |

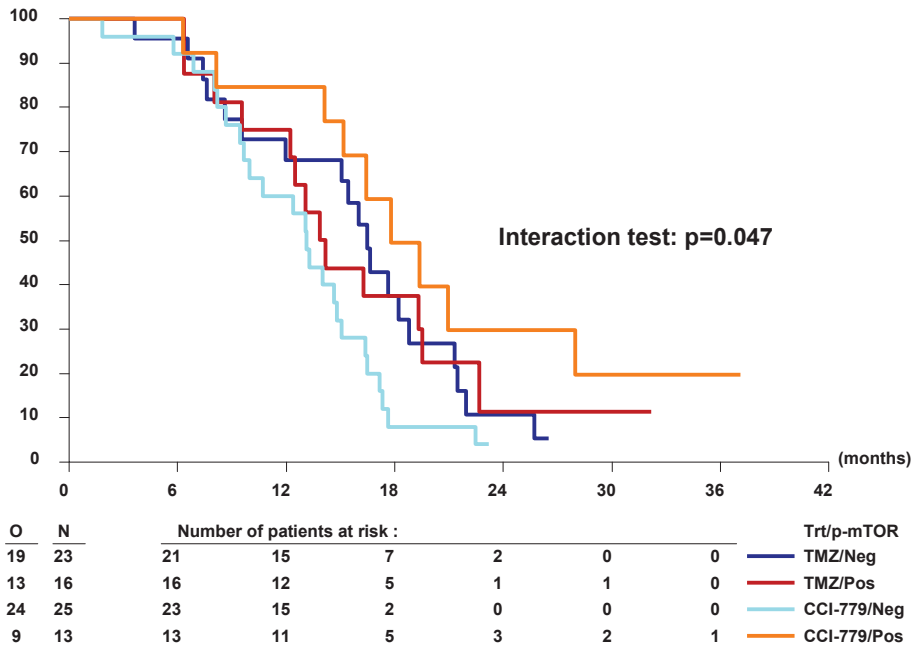
**B**




## Survival Time

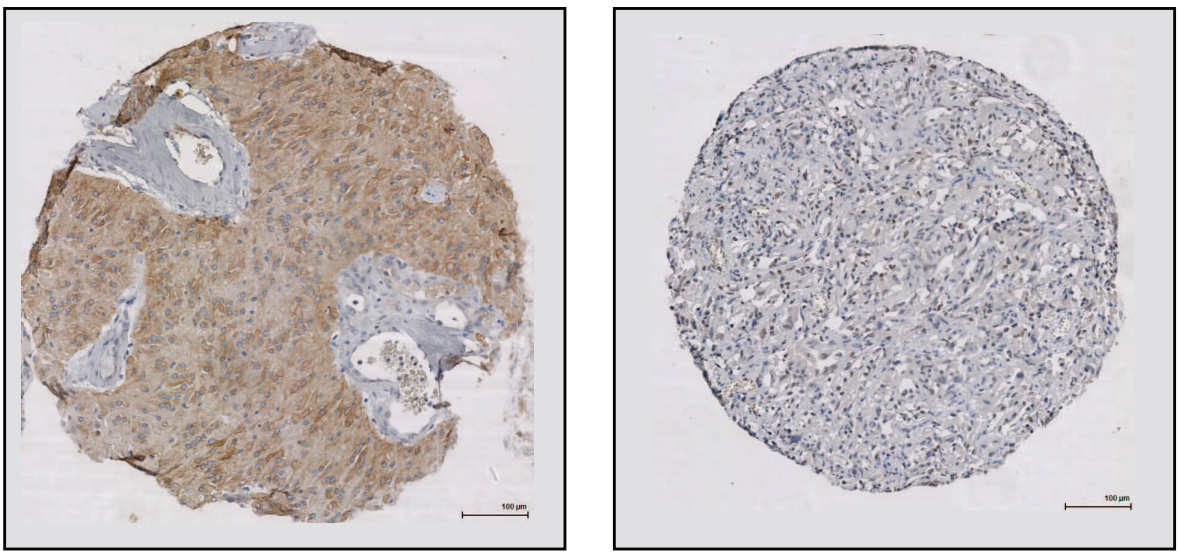
| Treatment | Patients (N) | Observed Events (O) | Hazard Ratio (95% CI) | P-Value (Log-Rank) | Median (95% CI) (Months) | % at 0.5 Year(s) (95% CI) |
|---|---|---|---|---|---|---|
| **TMZ** | 55 | 51 | 1.00 | 0.2358 | 5.95 (3.25, 8.02) | 50.00 (36.12, 62.39) |
| **CCI-779** | 56 | 54 | 1.26 (0.86, 1.86) | | 5.36 (3.71, 6.14) | 38.67 (25.96, 51.20) |

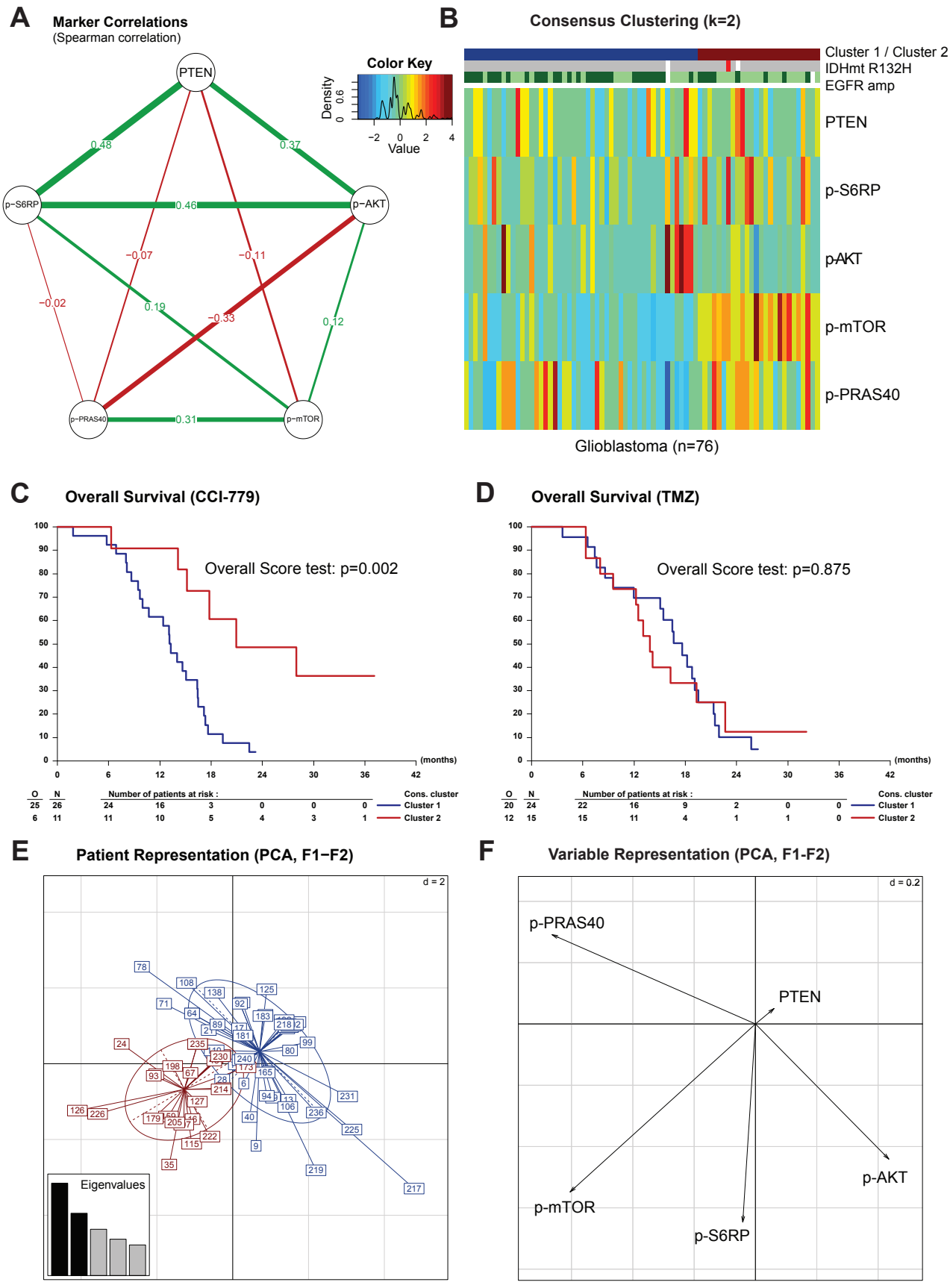
**A**




| Survival Time | | | Non-parametric | | Cox model | |
|---|---|---|---|---|---|---|
| treatment/p-mtor | Patients (N) | Observed Events (O) | Median (95% CI) (Months) | % at 2 Year(s) (95% CI) | Hazard Ratio (95% CI) | P-Value (Score test) |
| TMZ/p-mTOR Neg | 23 | 19 | 16.46 (9.53, 18.79) | 10.7 (1.8, 28.7) | 1.00 | 0.042 (df=3) |
| TMZ/p-mTOR Pos | 16 | 13 | 14.01 (9.56, 19.55) | 11.3 (0.9, 36.4) | 0.99 (0.49, 2.01) | |
| CCI-779/p-mTOR Neg | 25 | 24 | 13.11 (9.66, 15.08) | 4.0 (0.3, 17.0) | 1.71 (0.93, 3.14) | |
| CCI-779/p-mTOR Pos | 13 | 9 | 17.77 (14.09, 27.99) | 29.7 (7.4, 56.8) | 0.59 (0.26, 1.32) | |
| | | | | | Log-rank test: | p-value=0.041 |

**B**





AACR American Association for Cancer Research

Clinical Cancer Research

# Phase II study of radiotherapy and temsirolimus versus radiochemotherapy with temozolomide in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation (EORTC 26082)

Wolfgang Wick, Thierry Gorlia, Pierre Bady, et al.

*Clin Cancer Res* Published OnlineFirst May 3, 2016.